EFFECTS OF ALSIKE CLOVER/*RHIZOBIUM* SYMBIOSIS ON LODGEPOLE PINE SEEDLINGS AND SOIL NITROGEN IN WEST CENTRAL BRITISH COLUMBIA

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ABSTRACT

Losses of site nitrogen have been reported in British Columbia following prescribed burning and mechanical blading treatments used for site preparation in reforestation. While these treatments often lead initially to improved tree seedling establishment and survival, amelioration of nitrogen losses may be necessary to sustain long-term site productivity. Inoculated *Trifolium hybridum L.* (alsike clover) was seeded at O, 10, 20, and 30 kg/ha with *Pinus contorta* Doug, *ex* Loud (lodgepole pine) seedlings to determine 1) the effects of site preparation on infection and effectiveness of the clover-*Rhizobium* symbiosis and clover cover, and 2) the effects of the *clover-Rhizobium* symbiosis on survival, early growth, and foliar nitrogen concentration of the lodgepole pine seedlings and on soil nitrogen content and availability. The nitrogen-fixing symbiosis successfully established in all treatments, and site preparation (broadcast burn, windrow burn, and mechanical forest floor removal) had no significant effect on clover percent cover. After four growing seasons, the symbiosis had no effect on survival or height and diameter incremental growth of the seedlings. However, both needle mass and foliar nitrogen concentrations were significantly greater in clover-seeded plots compared to controls. There were significant increases in forest floor total and rnineralizable nitrogen as rate of seeding increased, although a similar effect was not detected for the mineral soil layer.

Effets de la symbiose du trèfle alsike et du Rhizobium sur les plants de pin et le contenu en azote du sol dans le centre-ouest de la Colombie-Britannique

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En Colombie-Britannique, des pertes en azote sont rapportées dans les sols suite aux traitements par feu et coupes mécaniques prescrits pour la préparation d'un site au reboisement. Il est reconnu que les bénéfices, à court terme, de ces traitements, conduisent initialement à l'amélioration de l'implantation des pousses d'arbres et de leur chances de survie. Cependant, la prévention des pertes d'azote pourrait également être nécessaire pour maintenir le rendement à long terme du site. Des pousses de Trifolium hybridum L. (trèfle hybride ou alsike) ont été plantées à raison de O, 10, 20 et 30 kg/ha avec du Pinus contorta Doug, ex Loud (variété de pin de l'ouest canadien) pour déterminer les effets de: 1) la préparation de site sur l'infection et l'efficacité de la symbiose trèfle-<u>Rhizobium</u> et du revêtement de trèfle; 2) la symbiose trèfle-Rhizobium sur la croissance initiale, la survie et la concentration d'azote dans les aiguilles de pousses de pin, ainsi que sur la chimie de l'azote du sol. La symbiose de fixation d'azote s'est bien établie et ce, pour tous les types de traitement. Aucun effet important ne fut détecté pendant la préparation du site (lors de différents types de feux et de l'enlèvement mécanique du tapis forestier) sur le pourcentage du revêtement de trèfle obtenu. Après quatre saisons de croissance, la symbiose n'avait plus d'influence sur la survie ou sur la hauteur ou le diamètre de croissance des pousses. Cependant, la masse des aiguilles ainsi que leur concentration d'azote étaient beaucoup plus élevées dans les espaces semés au trèfle que dans les secteurs-références. En plus, le sol forestier, dans les endroits brûlés avec la méthode "broadcast", possédait de plus grandes quantités d'azote total et minéralisable, à mesure que le taux d'implantation était augmenté.

Nitrogen (N) is the principal nutrient limiting the productivity of forest crops in temperate North America. It is also readily lost from soils as a consequence of volatilization, leaching, denitrification, runoff and erosion, as well as through biomass removal during harvesting, site preparation, and stand tending. Losses of site N have been reported in British Columbia following prescribed burning and mechanical blading treatment used for site preparation in reforestation (Brockley *et al.* 1991). Fortin *et al.* (1984) concluded that 30-80 kg/ha/yr of N will need to be replaced to maintain growth and site productivity in some situations. In natural ecosystems, N inputs may be derived from small amounts deposited in rain water, and by living organisms through biological N fixation. The conventional approach to maintaining site nutrients has been through the use of fertilizers, but concerns have arisen over the cost of fertilization and the effects of extensive synthetic fertilizer use on soil and ground water quality (Pritchett 1979). Consideration of forest management over the long term suggests the need for alternative methods of enhancing fertility.

Management of biological N fixation in forestry has been considered for many years (Assmann 1970, Beuter 1979, Haines and DeBell 1979, Rehfuess 1979, Jorgensen 1980, Granhall 1981, Fortin *étal.* 1984, Binkley 1986). The primary interest in the *\egume-Rhizobium*symbiosis in forestry has been for the amelioration of the inherent low N status of many forest ecosystems, or to address N losses caused by some forest management practices (Haines and DeBel1979, Fortin *et al.* 1984). Before that amelioration can be achieved, the legume and tree seedling crops must be established in a regime that is compatible with forest management objectives.

In soils that are low in N, such as forest soils following severe fire, or extreme mechanical disturbances, recolonization by N-fixing organisms is the primary means of restoring N fertility over time (Burns and Hardy 1975). However, actual N fixation rates are difficult to measure. Non-symbiotic N fixation was reported by Fortin *et al.* (1984) to be 2-5 kg/ha annually, which generally agrees with estimates reported by Binkley (1986) of 0-4 kg/ha annually. Actinorhizal plants have been reported to provide 0-150 kg/ha of fixed-N annually (Binkley 1986). Estimates of N fixed by legumes in forest plantations vary widely, Binkley (1986) reported some results ranging from 35 to 200 kg/ha annually. Gadgil (1979) reported that lupines provided 60 kg/ha annually under *Pinus radiata* (D.Don) until canopy closure. Jorgensen (1980) suggested that legumes in forest management should provide 50-100 kg/ha annually of fixed-N for three to five years to provide an economic return. Whatever the actual fixation rate is on any given site under the prevailing environmental conditions, biological N fixation has the potential to enhance site N-status, and to ameliorate losses of N resulting from forestry practices.

Legume species are of particular interest in forest management because many of the species' seeds and host-specific inoculants (*Rhizobium* spp.) are commercially available, which allows for controlled introduction directly into the reforestation prescription. In addition, because there are a number of species to choose from, a specific species (or mixes) can be chosen based on propagation and growth characteristics that match a site's environmental conditions and the silvics of the crop tree(s).

In their review of the subject, Fortin *et al.* (1984) found little literature dealing specifically with the effects of legumes on tree growth in North America. Most of the information they found came from experiments designed for other purposes. This study describes the establishment of the alsike clover (*Trifolium hybridum L.*)-*Rhizobium* symbiosis and it's effect on early growth, survival, and foliar N status of lodgepole pine (*Pinus conforta* Doug, *ex* Loud), and on soil N content and availability.

STUDY AREA AND EXPERIMENTAL DESIGN

The study area was located in west central British Columbia within one dominant ecosystem - the mesic bunchberry-moss site series of the Moist Cold Sub-boreal Spruce biogeoclimatic subzone (Pojar *et al.* 1984). The climate is characterized as a cold sub-boreal continental humid type, with severe, snowy winters and relatively warm, moist, and short summers.

Soils are dominantly Brunisolic Gray Luvisols (Agriculture Canada Expert Committee on Soil Survey 1987) developed on morainal blankets of deep (> 1 m) glacial till. The soil textures are loam

to clay loam, containing 20-35% coarse fragments. The site is gently to moderately sloping with south to southeast aspect.

The experiment was established as a split-plot design (Hicks 1982) with site preparation treatments as the main plot factor [broadcast burn, windrow burn, and mechanical forest floor removal (areas between windrows]. Each main plot was split into four 20 m² plots (4x5 m), replicated in three rows in each of three blocks. These split-plots were randomly assigned one of four rate of seeding levels (ROS treatment). The ROS treatment consisted of O (control), 10, 20, and 30 kg/ha of inoculated alsike clover seed. There were 108 split-plots in total. Treatment effects were tested using SAS 'General Linear Model' procedures (SAS Institute Inc. 1985) for planned contrasts of linear effects (Hicks 1982) due to RpS treatment, and Tukey's Studentized Range Test (HSD) (Zar 1984) to compare levels of site preparation treatment. Early effects of site preparation treatments on lodgepole pine seedlings have been reported by Blackwell (1989).

MATERIAL AND METHODS

Alsike clover and Rhizobium

The selection of alsike clover was based on local experience and on the acid and drought tolerance of commercial cultivars. Since *Rhizobium* strains are known to have different infectivity and effective N-fixing responses based on environmental conditions (Turvey and Smethurst 1983), nodules from alsike clover plants growing in the study area were collected, and the endophyte isolated and tested for infectivity and effectiveness in a pot experiment (Trowbridge 1987) using the basic techniques described in Vincent (1970). Six individual colonies of effective *Rhizobium leguminosarum* biovar *trifolii* were ultimately selected and cultured for use in the experimental "mixed-strain" inoculant. Strains were cultured in yeast extract mannitol broth and combined prior to addition to packages of sterile ground peat to prepare the inoculant (Vincent 1970).

Seed was weighed to correspond to ROS treatment levels, and each split-plot allocation of seed was then coated with the peat inoculant and calcium carbonate using procedures described in Trowbridge and HoII (1989). Seeding took place one day later, in early June, 1987.

The infection of alsike clover by *Rhizobia* was assessed when clover biomass was at its peak in each of the first four growing seasons (mid-August of 1987, 1988, 1989, and 1990). Individual plants were excavated from each split-plot, the roots gently agitated in water, and visually assessed for nodulation abundance and pigmentation. Module abundance was placed into one of four categories: none, few, many, and abundant. Nodules from the roots of these plants were cut open to determine the presence of leghemoglobin, a protein required during active N fixation in legumes indicated by pink pigmentation (Nash and Schuiman 1976). Pigmentation was assessed as present or absent.

Lodgepole pine

In early June, 1987 prior to alsike clover seeding, twelve lodgepole pine seedlings (2-11 plugs) were planted in each split-plot at 1 x 1 m spacing (1296 seedlings in total). Height and diameter were measured at the time of planting, and mid-August of each year. Differences between the 1987-88,1988-89, and 1989-90 growing seasons were calculated as incremental growth. Survival was assessed each year.

Foliar N status was assessed in the second and fourth growing seasons (1988 and 1990) by comparing treatment effects on needle total N concentration (%) levels. One lateral branch, from current year's growth on the second whorl of each surviving tree, was clipped and bulked to produce a composite sample for each split-plot. The needles were oven-dried at 70° C and mean needle mass (g dry weight/100 needles) was determined by averaging the masses of three subsamples of 1 needles each for each split-plot. The needles were analyzed for total N (CHN-analyzers, Carlo Erba Model 1106 at the Department of Oceanography, University of British Columbia, in 1988; and Leco Model CHN-600 at the Ministry of Forests Analytical Laboratory, Victoria, British Columbia, in 1990).

Soil nitrogen

Soil sampling for total N took place in May 1987 just prior to clover seeding (pre-treatment), and again in early June 1988 and 1990 (one- and three years post-treatment) for total N and mineralizable N. In all cases, the sample locations were randomly chosen from areas considered to represent acceptable planting spots, avoiding standing water, excessive coarse fragments, and large accumulations of decaying wood. The samples for chemical analysis were air-dried at room temperature until mass became constant, then sieved to pass a 2-mm mesh. The samples taken for determination of mineral soil bulk density and forest floor mass were oven dried at 105^oC until a constant mass was reached.

Chemical analysis was conducted on one sample per experimental unit (108 units) on each of forest floor and mineral soil. Each sample was a composite of four randomly located subsamples. The forest floor subsamples were each taken from 20 x 20-cm areas in the broadcast burned plots. Forest floor was not present in the windrowed or scraped areas, except in distinctive microsites. The composite mineral soil sample was taken from the undisturbed mineral soil interface to a depth of 15 cm using an auger. In the windrowed areas, the undisturbed mineral soil layer was commonly several centimetres below a layer of ash. Ash was not sampled. Soil N was measured using a Leco CHN-600 analyzer. Mineralizable N was estimated through a two week anaerobic incubation at 30^oC, followed by a 1N KCI extraction and colorimetric analysis for ammonium N. Analyses were done at the British Columbia Ministry of Forests Research Laboratory.

RESULTS AND DISCUSSION

Alsike clover-Rhizobium establishment

Infectivity of the inoculant and effectivity of N fixation were assessed as excellent. In the sampling scheme, infection was to be assessed by assigning one of four classes of nodulation (ranging from none to very abundant). However, nodulation was very abundant on all samples in all years. Pink pigmentation (presence of leghemoglobin) was observed in all nodules cut open in each year.

The mean percent cover estimates for levels of ROS treatment are illustrated in Figure 1. In the first growing season there were many individual plants in each plot, but percent cover was very low. There was a large increase in percent cover in subsequent years, followed by a reduction in 1990. The reduction in cover observed in the fourth growing season was attributed to natural dieback of the clover. Alsike clover is a short-lived perennial, living four to six years in a favourable environment (Martin, *et al.* 1976), but is usually managed as a biennial (Walton 1988). There was no evidence of clover plants in the control plots in any year, although it was observed that re-seeding and new establishment took place in the seeded plots in 1989 and 1990. What level of cover these new clover plants will attain is unknown at this time.

ROS treatment had a highly significant linear effect (p < 0.01) on percent cover in each year, which may be attributed to the large difference in values between control (always 0.0) and clover-seeded plots. However, the actual differences among the seeding rates were only a few percent in most years. It appeared that seeding rate made little real difference in percent cover - by the third growing season clover occupied a large amount of space in the plots regardless of the initial seeding rate.

Effects of site preparation treatment were not significant for clover cover [Tukey's Studentized Range Test (HSD), p < 0.05] in any year. However, analysis of variance did show slightly significant main effects in the third (1988) and fourth (1990) growing seasons (Table 1). In these years, the windrow burn treatment had slightly higher mean cover compared to broadcast and forest floor removal, but there was not a consistent trend for the latter treatments.





Figure 1. Alsike clover percent cover by year.

Table 1.	Response	of	alsiko	clover	00110		·		
			aisike	clovel	cover	ιo	site	preparation	treatment.

	Site preparation			Main effect	
	Broadcast burn	Windrow burn	Forest Floor removal	Pr > F	
Percent cover:					
1987 1988 1989 1990	1 (0.3) ¹ 25 (3.9) 56 (5.8) 42 (4.8)	3 (1.8) 29 (3.8) 61 (6.1) 52 (5.7)	3 (0.5) 31 (3.5) 54 (5.4) 50 (5.8)	0.5052 0.4248 0.0685 0.0486	
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1 standard error in parentheses

ROS treatment had no significant effect on total or incremental height in any year (Table 2). The overall mean total height of seedlings at the end of the 1990 growing season was 70.9 cm, and incremental height growth was 27.5 cm for 1989-90. The leaders of the seedlings had over-topped the clover in most cases by 1989, and in 1990 the leader and one to two whorls were above the clover canopy. In succeeding years, pine seedlings will begin to shade the clover.

ROS treatment had significant effects on total and incremental diameter as shown in Table 2. However, the actual differences measured were very small. In relative terms, this effect represented a 13% decrease (0.4 mm) in diameter increment between no clover and 34-42% clover cover in the second growing season. In the third growing season, at which time the clover cover ranged from 74-78%, the increment difference represented a 20% decrease (0.9 mm) between control and clover-seeded plots. However, by the end of the fourth growing season (1989-90), differences in diameter increment among levels of ROS treatment were no longer significant., Seedling diameter, averaged across all treatment levels, increased by 5.8 mm during this period.

		Linear effect:			
	0	10	20	30	Pr > F
Total height (cm):					
1987	16.8 (0.4)	16.2 (0.4)	16.6 (0.4)	16.3 (0.5)	0.5346
1988	25.5 (0.6)	24.3 (0.7)	24.4 (0.9)	24.8 (0.7)	0.4310
1989	43.2 (1.7)	42.7 (1.5)	43.3 (1.8)	43.0 (1.7)	0.9663
1990	72.3 (2.9)	70.1 (3.1)	70.1 (3.4)	71.1 (3.2)	0.5524
Height increment (cm):					
1987-88	8.6 (0.5)	8.1 (0.6)	7.6 (0.7)	8.5 (0.6)	0.6658
1988-89	17.8 (1.2)	18.3 (1.1)	18.9 (1.3)	18.2 (1.2)	0.5594
1989-90	28.7 (1.5)	27.3 (1.6)	26.9 (1.7)	27.1 (1.5)	0.1307
Total diameter (mm):					
1987	3.5 (0.1)	3.6 (0.1)	3.6 (0.1)	3.5 (0.1)	0.7373
1988	6.7 (0.2)	6.4 (0.2)	6.3 (0.2)	6.3 (0.2)	0.0102
1989	11.3 (0.4)	10.0 (0.4)	10.1 (0.5)	9.8 (0.4)	0.0002
1990	17.3 (0.8)	15.7 (0.7)	15.7 (0.8)	16.0 (0.8)	0.0287
Diameter increment (m	nm):		1283201		0.0000
1987-88	3.1 (0.2)	2.8 (0.2)	2.7 (0.2)	2.7 (0.2)	0.0022
1988-89	4.6 (0.3)	3.6 (0.3)	3.8 (0.3)	3.6 (0.3)	0.0010
1989-90	6.0 (0.4)	5.6 (0.4)	5.7 (0.4)	6.0 (0.4)	0.8132
Survival (%):				~ (0,0)	0.7004
1988	80 (3.4)	83 (2.8)	81 (3.2)	82 (3.2)	0.7924
1989	78 (3.1)	79 (3.2)	77 (3.7)	79 (4.2)	0.9570
1990	78 (3.0)	79 (3.2)	75 (4.0)	// (3./)	0.4154
Needle mass (g/100):			0.00 (0.05)	0.00 (0.05)	0.0004
1988	1.05 (0.05)	0.93 (0.05)	0.89 (0.05)	1 46 (0.05)	0.0024
1990	1.30 (0.06)	1.43 (0.05)	1.44 (0.00)	1.40 (0.00)	0.001
Foliar N (%):		4 47 (0.00)	1 42 (0.04)	1 49 (0 03)	0.6633
1988	1.46 (0.04)	1.47 (0.03)	1.43 (0.04)	1 39 (0.02	0.0001
1990	1.23 (0.03)	1.37 (0.02)	1.00 (0.02)	1.00 (0.02	,

Table 2. Response of lodgepole pine seedlings to rate of seeding treatment.

¹ standard error in parentheses

The initial reduction in seedling diameter growth may be attributed to partial shading from clover. Since the lodgepole pine seedlings had grown well above the clover canopy by the fourth growing season, any shading effect of clover on seedlings to date will likely be reversed in subsequent years.

ROS treatment had no effect on survival in any year (Table 2). Overall survival across ROS treatment was 82% in 1988, 78% in 1989, and 77% in 1990.

No previous studies reported in the literature have investigated interplanting of alsike clover and lodgepole pine. The existing literature on legumes in forestry is, however, overwhelmingly supportive of the observation of increased growth of associated trees. Finn (1953) found increased height and diameter growth of several deciduous trees on all but one of his sites when the trees were associated with *Robinia pseudoacacia* L. (black locust). Haines *et al.* (1978) found increased height and volume (two-and threefold respectively) growth of *Platanus occidentalis* L. (sycamore) following establishment of clovers and vetch. Assmann (1970) summarized a study by Wiltch (1954) demonstrating increased height and a 60% volume increase over a 10-year period in *Pinus sylvestris* L. (Scots pine) grown in association with lupine. Rehfuess (1979), in examining these results, reported overall growth reduced in lupine treatments for the first three or four growing seasons followed by improved growth after an initial lag period (3-7 years). The Scots pine experiments are confounded, in part, by simultaneous cultivation and other nutrient amendments. Kumi (1986) reported no overall significant effect of associated legumes on juvenile height growth of *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) except on poor sites, where there was a 13% increase.

Needle mass and N status

At the end of the second growing season (1988), ROS treatment had a significant negative effect on needle mass, however foliar N concentration was similar among the treatments (Table 2). By the end of the fourth growing season (1990), ROS treatment on both needle mass and foliar N concentration was found to have significant positive linear effects (Table 2). The overall higher foliar N values observed earlier (1988) in the experiment are attributed to carry-over from fertilization in the nursery before outplanting. Concentration levels of 1.5% (w/w) or higher foliar N for lodgepole pine are considered by Ballard and Carter (1985) to be adequate. In interior British Columbia, levels much lower than this are commonly reported (Brockley 1989, Yole *et al.* 1990). It appeared that, by the fourth growing season (1990), foliage N concentrations had begun to reflect slight to moderate N deficiency, however the seedlings associated with the clover-seeded plots clearly had significantly greater concentrations compared to controls.

According to Jorgensen (1980), trees respond initially to N fixed by legumes by increasing foliar N. Finn (1953) found greater total foliar N for trees associated with black locust cover compared to controls in almost every instance. Haines *et al.* (1978) found higher foliar N concentrations in Sycamore associated with legumes, as did Jorgensen (1980) in *Liquidambar styraciflua L.* (sweetgum) and *Pinus taeda* L. (loblolly pine) foliage and Gadgil (1979) in *Pinus radiata* D. Don (Monterey pine).

Soil N content and availability

Results are presented in Table 3. For total N, no significant linear effects were observed in the forest floor or mineral soil after two years of clover establishment. After three years (1990), there was a significant increase in total N in the forest floor, but a similar effect was not detected in the mineral soil layer. These results may be attributed to newly fixed N in the aboveground clover detritus accumulating on the soil surface as litter, and being decomposed and recycled within the forest floor. After three years, the forest floor in clover seeded plots contained 17% (53 kg) more total N compared to controls.

Increased levels of forest floor mineralizable N were observed after one and three years of litter contribution in clover-seeded plots (1988 and 1990). The effect was very small in 1988, however by 1990 there was a clear and large difference between clover-seeded plots and controls. On average, there were 16 kg/ha more mineralizable N in clover-seeded plots in 1990. Mineralizable N, as a result of anaerobic incubation, is an index of N availability (Page *et al.* 1982), and although these amounts appear small relative to total N, this "available N" is considered to represent the primary source of N

for most plants. In comparing the percent of nnineralizable N to total N, among control and cloverseeded plots, the results were 1.5% and 5.0% respectively. This demonstrates a substantial improvement in the form of N released in the forest floor of clover-seeded plots compared to controls.

ROS treatment had a significant effect on mineralizable N in the mineral soil in 1988, but not in 1990. It is unclear why the belowground clover detritus has not been observed to contribute substantially larger amounts of mineralizable or total N to the upper mineral soil. More sampling and further investigation may provide a better understanding in future measurements.

Table 3. Response of soil nitrogen content (kg/ha) to rate of seeding.

			Seed rate (kg/ha):				Linear contrast:	
		<u>0</u>	<u>10</u>	20	30	<u>Pr > F</u>		
Total N:	1 I 201	1000 - 1000 - 1000 1000 - 1000 - 1000 1000 - 1000 - 1000			9			
forest floor	1987 1988 1990	246 (21) ¹ 247 (12) 267 (15)	240 (19) 242 (14) 310 (23)	227 (17) 255 (15) 328 (20)	234 (16) 242 (15) 322 (21)	0.2354 0.9424 0.0213		
mineral	1987 1988 1990	870 (81) 940 (111) 703 (89)	902 (96) 832 (112) 620 (67)	906 (81) 850 (94) 793 (93)	856 (88) 800 (109) 661 (77)	0.8116 0.1424 0.8049		
Mineralizable	N:							
forest floor	1988 1990	3 (0) 4 (1)	3 (1) 14 (2)	3 (1) 17 (2)	4 (1) 17 (2)	0.0448 0.0001		
mineral	1988 1990	26 (5) 23 (4)	26 (6) 24 (3)	28 (5) 25 (3)	30 (7) 22 (3)	0.0241 0.6455		

¹ standard error in parentheses

SUMMARY

The experimental endophyte's ability to infect alsike clover and cause effective N fixation, and the establishment of alsike clover on the site, were assessed as excellent. Clover percent cover significantly increased with increasing rate of seeding. Site preparation treatments had no significant effect on the establishment of the symbiosis or clover cover.

The symbiosis had no effect on height or survival of lodgepole pine. Initially, rates of seedling diameter growth decreased with increasing ROS levels. However, by the end of the fourth growing season differences in diameter increment were no longer significant. Needle mass was significantly less after two growing seasons where the seedlings were associated with clover compared to controls, but this trend was reversed in the fourth growing season when both needle mass and foliar N were significantly greater as rate of seeding increased.

After three years, total N and mineralizable N had increased significantly in the forest floor of clover-seeded plots. This was attributed to the newly fixed N in aboveground clover detritus, that

accumulated on the soil surface and had begun to incorporated into the forest floor ecosystem. Similar increases have not yet been detected in the upper mineral soil.

In the first four years of the study, both alsike clover and lodgepole pine have become established and are growing well together. Furthermore, there appears to be a benefit from the clover symbiosis to lodgepole pine seedling growth in significantly increased needle mass and foliar N status. Appraisal of this site will continue to determine if these early results remain the same, disappear, or become increasingly significant as the crops mature while sharing and/or competing for site resources.

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